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Gyrodactylus eyipayipi sp. n. (Monogenea: Gyrodactylidae) from *Syngnathus acus* (Syngnathidae) from South Africa

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Abstract: *Gyrodactylus eyipayipi* sp. n. is described from the skin, gills, flute and male brood pouch of captive specimens of the greater pipefish *Syngnathus acus* L., collected for and maintained at the Two Oceans Aquarium in Cape Town, South Africa. It is the first marine *Gyrodactylus* species reported from the African continent. The new species is compared to the three known *Gyrodactylus* species affecting syngnathiform hosts (*G. pisculentus* Williams, Kritsky, Dunnigan, Lash et Klein, 2008, *G. shorti* Holliman, 1963, and *G. syngnathi* Appleby, 1996). Although all four species have similar-sized and shaped attachment hooks with some overlap, separation of the species is possible using marginal hook morphology. The marginal hooks of *G. eyipayipi* measure (mean) 30 µm in total length and are larger than those of the three other species (mean, 24–28 µm). *Gyrodactylus eyipayipi* can also be discriminated based on differences in the shape of the marginal hook sickle notably by its long sickle point which extends far beyond the toe, its blunt rounded toe and, by the approximate rectangular shape to the base of the sickle. By comparison, the sloping toe regions of *G. pisculentus* and *G. syngnathi* give the sickle bases an approximately triangular shape, whilst the short sickle point and open aperture to the sickles of *G. shorti* allow for their discrimination from *G. eyipayipi*.

Keywords: Monogenea, *Gyrodactylus eyipayipi*, *Syngnathus acus*, pipefish, public aquarium, South Africa

The greater pipefish *Syngnathus acus* L., like all pipefish, are prized as aquaria species because of their interesting body shape, but also because syngnathids, generally, breed successfully under captive conditions and have basic husbandry requirements for their maintenance. Within South African coastal waters, *S. acus* is a common species and is reported from the west coast to northern Natal, in estuaries and in deeper waters down to 110 m (Dawson 2003). Yet despite the wide geographic range of *S. acus*, which extends from the Norwegian Atlantic coast, down the west coast of Africa round to Zululand on South Africa's east coast, only one parasitology-based study is known from the literature. Longshaw et al. (2004) conducted a histological examination of *S. acus* collected from the Mersey Estuary, UK, and although a number of metazoan and prokaryotic infections were recorded, no monogeneans were found.

The genus *Gyrodactylus* von Nordmann, 1832 contains more than 400 species (Harris et al. 2004); the vast majority of these are reported from Eurasia, the Neotropic and Nearctic ecozones, whilst only a few species are described from North Africa and the Afrotropic regions. There are no records from African marine coastal waters and only 21 species are currently known from African freshwater environs (Christison et al. 2005, Nack et al. 2005, Přikrylová et al. 2009). Three species of *Gyrodactylus* from syngnathid hosts are known, namely: *G. pisculentus* Williams, Kritsky, Dunnigan, Lash et Klein, 2008 from a population of northern pipefish, *Syngnathus fuscus* Storer, held in Woods Hole Science Aquarium (Williams et al. 2008), *G. shorti* Holliman, 1963 from the Gulf pipefish, *Syngnathus scovelli* (Evermann et Kendall) (see Holliman 1963), and *G. syngnathi* Appleby, 1996 from a Norwegian population of Nilsson's pipefish, *Syngnathus rostellatus* Nilsson (see Appleby 1996).

The current study describes a new species of *Gyrodactylus* collected from a South African population of *S. acus*, the first *Gyrodactylus* species to be described from the African marine environment.

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MATERIALS AND METHODS

Specimen collection and preparation. Fifty *S. acus* were collected from False Bay at "Long Beach" near Simon's Town, Cape Town (34°11′12.90″S, 18°25′38.51″E) for use in the public exhibition at the Two Oceans Aquarium. All the pipefish were screened for the presence of parasites during a mandatory quarantine period prior to public display. A sub-sample of 10 pipefish were placed individually into glass inspection bowls containing fresh 35 ppt seawater and observed under an Olympus SZ60 stereo zoom dissection microscope fitted with a fibre-optic cold light-source.

The pipefish were subsequently humanely euthanased by severing the spinal column just behind the opercula with a scalpel blade and then dissected placing the gills, flute, body (entire external surface) and the male brood pouch contents into separate labelled Petri dishes. Prior to gill and flute dissection, any gyrodactylids attached to the external surface of the head were removed. Eight of the *S. acus* were female (134 ± 14.1 (114–150) mm long; 0.9 ± 0.2 (0.5–1.1) g) and two were male (268 ± 5.7 (264–272) mm long; 5.9 ± 0.4 (5.6–6.2) g). The number of gyrodactylids recovered from each region of the host were determined and then fixed in absolute ethanol (AR-grade absolute). The ethanol-fixed specimens were then rehydrated in distilled water before being mounted whole in glycerine jelly (Gussev 1983) for morphometric analysis.

The haptors of an additional 20 fresh specimens were excised, placed individually on glass slides and then digested using the proteolytic method given in Harris et al. (1999). The tissue-free haptoral sclerite preparations were then mounted in ammonium picrate glycerine and photographed. The bodies corresponding to each haptor were then fixed in 95% ethanol and stored in individual 0.5 ml Eppendorf tubes at −20 °C until they were processed for molecular characterisation.

Morphometric analysis. A total of 73 whole specimens and 20 haptor digest preparations were mounted in glycerine jelly and used for morphometric analysis. Measurements of the haptoral armature (presented in µm and degrees) follow the protocol for *Gyrodactylus* species given in Shinn et al. (2004) and are presented in Table 1 as the mean ± standard deviation followed, in parentheses, by the range and the number of specimens that were measured. Morphometric measurements were taken using Olympus AnalySIS5® analysis software through an Olympus CX41 light microscope fitted with a phase-contrast and dark field condenser and an Olympus Altra20® digital camera.

Molecular analyses. Six of the 20 gyrodactylid bodies stored in ethanol were subjected to molecular analyses by sequencing a region spanning the ribosomal internal transcribed spacers 1 and 2 (ITS1 and 2) and 5.8S. This fragment is the most common molecular marker for species discrimination in the genus *Gyrodactylus* (see Matějusová et al. 2001, Ziętara and Lumme 2003). DNA was extracted from individual specimens using the QIAamp DNA Mini Kit (Qiagen) in accordance with the manufacturer's instructions. For PCR, the primer pairs ITS1A and ITS2 (Matějusová et al. 2001) were used to amplify the specified fragment. PCR reactions were performed with puRe Taq Ready-to-Go PCR beads (Amersham Biosciences) in a GeneAmp PCR System 9700 (Applied Biosystems) using the following protocol: 4 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C and 2 min at 72 °C.

PCR-products were purified using a Macherey-Nagel NucleoSpin® Extract II and 10 µl reactions were subsequently sequenced on a MEGABACE 1000 (GE Healthcare) using DyeET-terminator mix (GE-Healthcare). Both PCR primers and the internal primers ITS1R, ITS2F, ITS18R and, in some instances, the ITS28F (Ziętara and Lumme 2003) were used for sequencing the full ITS fragment. Sequences were proof read in VectorNTI 10.3 (Invitrogen) and compared with sequences from available *Gyrodactylus* species via a GenBank BlastN search (http://www.ncbi.nlm.nih.gov/) (Zhang et al. 2000).

RESULTS

Examination of the eight female *S. acus* revealed they had an average 6.1 ± 3.9 (0–11) gyrodactylids on the body, 18.3 ± 19.0 (2–52) on the gills and 13.5 ± 9.3 (0–24) in the flute. By comparison, the two male *S. acus* had an average 45 ± 8.5 (39–51) gyrodactylids on the body, 2.5 ± 3.5 (0–5) on the gills, 10 ± 7.1 (5–15) in the flute, and, 17.0 ± 8.5 (11–23) within the brood pouch. Morphological examination of the haptoral armature of the 93 gyrodactylids revealed that they represented a single species which differed from the three already known species parasitising syngnathid hosts. Comparative measurements for the new species together with those for the three other *Gyrodactylus* species are presented in Table 1.

Gyrodactylus eyipayipi sp. n. Figs. 1A–E, 2, Table 1

Description. Based on the measurement of 73 coverslip-flattened specimens and 20 haptor digests. Total body length including haptor 443 ± 82.1 (286–629), width at uterus 125 ± 23 (76–173). Haptor roughly circular 63 ± 9.4 (47–99) long, 66 ± 9.5 (46–86) wide. Intestinal crura extending to the mid-point of testes. Anterior pharynx bulb 32 ± 4.8 (18–48) long, 52 ± 7.4 (34–70) wide. Posterior pharynx bulb 40 ± 6.1 (27–58) long, 74 ± 11.0 (51–96) wide. Male copulatory organ (MCO; Fig. 1A) posterior to posterior pharynx bulb, 16 ± 2.1 (10–19) in diameter. MCO armed with 1 principal spine, 2 smaller flanking spines and a single row of 5–9 smaller spines. Hamulus (Figs. 1B, C, 2) total length 45 ± 1.9 (39–49), shaft length 26 ± 1.9 (20–30), proximal shaft width 8 ± 0.8 (7–10), point length 20 ± 1.3 (15–22). Outer aperture angle 37 ± 2.8° (30–42°), inner aperture angle 49 ± 3.8° (40–56°), aperture length 15 ± 1.4 (12–18). Hamulus distal shaft width 6 ± 0.4 (5–7), root length 16 ± 1.6 (13–21) long. Dorsal bar simple (Fig. 1B) 12 ± 2.3 (8–20) long, 2 ± 0.4 (2–3) wide. Ventral bar with reduced processes (Fig. 1B), total length 16 ± 1.2 (14–19), 17 ± 0.9 (15–19) wide. Ventral bar process-to-mid length 2 ± 0.6 (1–4), median length 8 ± 0.8 (7–10). Ventral bar membrane reduced, approximately triangular (Fig. 1), membrane length 6 ± 0.9 (5–9). Marginal hook (Fig. 1C) total length 30 ± 2.0 (25–34), shaft length 23 ± 1.9 (18–28). Marginal hook sickle length 7 ± 0.3 (7–8), sickle proximal width 6 ± 0.3 (5–6), sickle distal width 7 ± 0.5 (5–8). Marginal

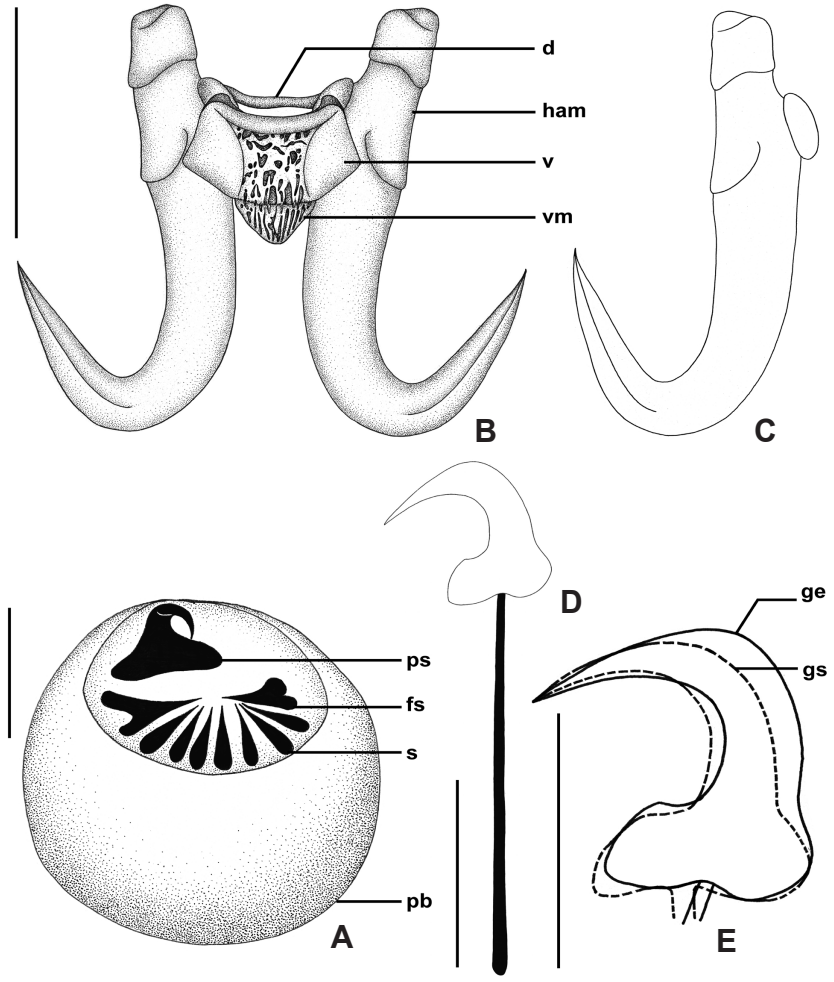

Fig. 1. *Gyrodactylus eyipayipi* sp. n. **A –** male copulatory organ; **B –** central armature; **C –** hamulus; **D –** marginal hook; **E –** marginal hook sickle overlay of *G. eyipayipi* sp. n. and *G. shorti* Holliman, 1963. *Abbreviations*: d – dorsal bar; fs – flanking spine; ge – *G. eyipayipi* (solid line); gs – *G. shorti* (dotted line); ham – hamulus; pb – male copulatory organ bulb; ps – principal spine; s – row of small uniform spines; v – ventral bar; vm – ventral bar membrane. Scale bars: A = 10 µm; B, C = 20 µm; D, E = 5 µm.

hook toe length 3 ± 0.3 (2–4), aperture 7 ± 0.6 (6–9), instep arch height 0.6 ± 0.1 (0.4–0.9).

Molecular characterisation. A 1212 bp PCR product covering ITS1 (658 bp), 5.8S (157 bp) and ITS2 (397 bp) was sequenced from five specimens and submitted to GenBank under accession number FJ040183. One additional specimen submitted under GenBank accession number FJ040184 was found to differ in its ITS1 sequence (657 bp) at three positions: a deletion at position 64, a transversion (A for a C) at position 176, and, a transition (A for G) at position 389. The nucleotide sequence of the 5.8S and the ITS2 regions for these six specimens were identical. A BlastN (Zhang et al. 2000) search in GenBank in June 2008 using the entire sequence revealed no identical or close hits. The molecular sequence obtained for *G. eyipayipi* represents the first sequence to be obtained from a gyrodactylid parasitising a syngnathid host.

Type host: Greater pipefish *Syngnathus acus* L. (Syngnathiformes: Syngnathidae).

Type locality: False Bay (34°11′12.90″S, 18°25′38.51″E) and subsequently maintained at Two Oceans Aquarium, Victoria & Alfred Waterfront, Table Bay Harbour, Cape Town, South Africa.

Site of infection: Gills, flute (internal surface), body (entire external surface) and lining of male brood pouch.

Type material: Collected October 2007. Holotype (BMNH 2009.2.27.1) and 3 paratypes (BMNH 2009.2.27.2-4) deposited in The Natural History Museum, London; 2 paratypes (SAMCTA 29463) and 50 paratypes (SAMCTA 29464) deposited in the Iziko South African Museum, Cape Town.

DNA reference sequences: Two sequences covering the internal transcribed spacer 1 and 2 and 5.8S are deposited in GenBank under Acc. Nos. FJ040183 and FJ040184.

Etymology: The species name is taken from the Xhosa word "*eyiphayiphi*" meaning "*it is a pipe*" after the host pipefish.

Remarks. *Gyrodactylus eyipayipi* is larger than *G. shorti* Holliman, 1963 in hamulus total length (45 ± 1.9 vs. 36), ventral bar total length (16 ± 1.2 vs. 14) and width

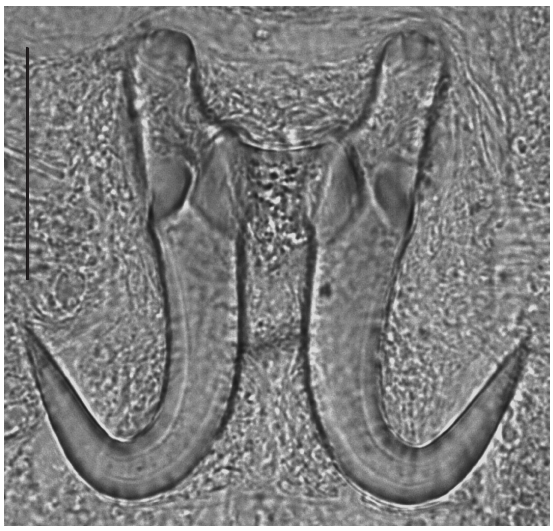

Fig. 2. *Gyrodactylus eyipayipi* sp. n., photomicrograph of central armature. Scale bar = 20 µm.

(17 ± 0.9 vs. 8) (Table 1). These two species can also be separated by the subtle differences in marginal hook sickle morphology which are presented in Fig.1E. When the outlines of these two hooks are overlaid, the sickle point of *G. eyipayipi* is seen to be much longer and the entire hook is comparatively more robust than that of *G. shorti*. The MCO of *G. syngnathi* is described as possessing a principal spine followed by a ring of smaller spines of unequal size. *Gyrodactylus eyipayipi*, by comparison, has a principal spine flanked by two smaller spines of equal size, followed by a ring of 5–9 smaller uniform spines, which is similar to the MCO of *G. pisculentus* illustrated in Williams et al. (2008). The MCO of *G. eyipayipi*, however, is wider than that of *G. pisculentus* (16 ± 2.1 vs. 13). The marginal hooks of *G. eyipayipi* are larger than those of *G. pisculentus* (30 ± 2 vs. 25) and can be separated by morphology; notably the shape of the base of the marginal hook sickle. The marginal hooks of *G. syngnathi* differ markedly from the remaining three species of *Gyrodac-*

Table 1. Morphological measurements (mean ± standard deviation followed by the range and number of specimens measured in parentheses, are provided in micrometres) of *Gyrodactylus eyipayipi* sp. n. from *Syngnathus acus* L. from False Bay, South Africa which are presented alongside three other species of *Gyrodactylus* known to parasitise syngnathiform hosts.

Structures measured	*G. eyipayipi* sp. n.*	*G. pisculentus* Williams et al. 2008	*G. shorti* Holliman 1963	*G. syngnathi* Appleby 1996
Total body length	443 ± 82.1 (286–629, n = 73)	335 (249–407, n = 23)	256 (176–360)	500 (400–560, n = 4)
Total body width	125 ± 23 (76–173, n = 73)	99 (75–126, n = 25)	84 (62–106)	110 (93–135, n = 4)
Haptor length	63 ± 9.4 (47–99, n = 59)	67 (52–72, n = 24)	53 (44–68)	–
Haptor width	66 ± 9.5 (46–86, n = 59)	65 (58–70, n = 23)	46 (37–54)	–
Posterior pharynx bulb length	40 ± 6.1 (27–58, n = 71)	–	32 (25–42)[1]	65 (64–67, n = 3)[2]
Posterior pharynx bulb width	74 ± 11 (51–96, n = 71)	53 (48–58, n = 25)	42 (31–52)[1]	78 (74–84, n = 3)
Anterior pharynx bulb length	32 ± 4.8 (18–48, n = 67)	–	–	–
Anterior pharynx bulb width	52 ± 7.4 (34–70, n = 67)	34 (31–40, n = 25)	–	46 (45–47, n = 3)
Male cop. organ (MCO) diam.	16 ± 2.1 (10–19, n = 51)	13 (10–15, n = 14)	10 (8–14)	(13–17, n = 2)
Number of MCO spines	8–12 (n = 51)	10–12	–	9–12
Hamulus (Ham) aperture	15 ± 1.4 (12–18, n = 63)	–	–	–
Ham proximal shaft width	8 ± 0.8 (7–10, n = 64)	–	–	–
Ham point length	20 ± 1.3 (15–22, n = 63)	–	–	19.4 (18–20)
Ham distal shaft width	6 ± 0.4 (5–7, n = 65)	–	–	–
Ham shaft length	26 ± 1.9 (20–30, n = 63)	–	–	32.2 (30–35.5)
Ham aperture angle (º)	37 ± 2.8 (30–42, n = 63)	–	–	–
Ham inner aperture angle (º)	49 ± 3.8 (40–56, n = 63)	–	–	–
Ham root length	16 ± 1.6 (13–21, n = 64)	–	–	15.5 (14–17.5)
Ham total length	45 ± 1.9 (39–49, n = 64)	41 (37–43, n = 26)	36 (34–37)	44.3 (41.5–47)
Ventral bar (Vb) total width	17 ± 0.9 (15–19, n = 52)	–	8 (7–9)	–
Vb total length	16 ± 1.2 (14–19, n = 48)	14 (13–16, n = 17)	14 (12–15)	16.4 (15–18)
Vb process-to-mid length	2 ± 0.6 (1–4, n = 51)	–	–	–
Vb median length	8 ± 0.8 (7–10, n = 52)	–	–	8.7 (8–9.5)
Vb membrane length	6 ± 0.9 (5–9, n = 48)	–	–	4.8 (4–5.5)
Marginal hook (Mh) total length	30 ± 2.0 (25–34, n = 61)	25 (21–28, n = 13)	24 (23–26)	27.7 (26–29)
Mh shaft length	23 ± 1.9 (18–28, n = 61)	–	–	22.2 (20.5–23.5)
Mh sickle length	7 ± 0.3 (7–8, n = 69)	7 (6–8, n = 26)	–	6 (6–6.5)
Mh sickle proximal width	6 ± 0.3 (5–6, n = 69)	–	–	4
Mh sickle distal width	7 ± 0.5 (5–8, n = 69)	–	–	5
Mh toe length	3 ± 0.3 (2–4, n = 69)	–	–	–
Mh aperture	7 ± 0.6 (6–9, n = 69)	–	–	–
Mh instep / arch height	0.6 ± 0.1 (0.4–0.9, n = 68)	–	–	–
Dorsal bar length	12 ± 2.3 (8–20, n = 50)	12 (10–14, n = 16)	–	–
Dorsal bar width	2 ± 0.4 (2–3, n = 50)	–	–	–

*Measurements are given to the nearest micrometre; [1]total pharynx length and width values (Holliman 1963); [2]total pharynx length value (Appleby 1996).

tylus considered here but can be separated from *G. eyipayipi* by the proximal width (4 vs. 6 ± 0.3) and distal width (5 vs. 7 ± 0.5) dimensions of the marginal hook sickle (Table 1).

DISCUSSION

Gyrodactylus eyipayipi represents the first *Gyrodactylus* species to be described from the marine environment from Africa and the first from a public aquarium from South Africa and follows the recent description of *G. pisculentus* from *S. fuscus* from the Woods Hole Science Aquarium in the United States (Williams et al. 2008). At present there are four *Gyrodactylus* species described from syngnathid hosts in addition to reports of *Gyrodactylus* spp. from the Bay pipefish, *Syngnathus leptorhynchus* Girard, from at least four major public aquaria in the United States (pers. comm. B. Christie, D. Powel, R. Burhans and R. Brynda). Unfortunately, none of the gyrodactylids parasitising *S. leptorhynchus* were retained for identification.

Gyrodactylus shorti and *G. eyipayipi* are the only two species to be collected from their respective male host pipefish's brood pouch while *G. syngnathi* was recovered from the head, body and fins only of its respective host *Syngnathus rostellatus* (Appleby 1996). *Gyrodactylus eyipayipi* is also the first species recorded from the internal surface of the flute. While *G. eyipayipi* appears to display a preference for the gills and flute in females, the two males that were examined in this study were observed to harbour larger intensities on the body surface. Although the advantage of such preference for site microhabitats on the different sexes is unclear, it is possible that courtship behaviour of the male *S. acus* lends itself as a successful mode of transmission through direct body contact with multiple females. It is possible that fry may become infected prior to their release from the male brood pouch, although, infection in young pipefish has not yet been confirmed.

Currently there are no reports of problematic *Gyrodactylus* species in South African public aquaria or South African marine aquaculture. Although all the *S. acus* hosts that were examined were found to be infected with *G. eyipayipi*, the parasite did not appear to have a significant impact on the health of the additional specimens of *S. acus* that were held in quarantine or were already on public display.

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